REMARKS

Amendments to the Specification

According to the foregoing amendments to the specification, the Statement as to Federally Funded Research has been amended. Applicants submit that no new matter has been introduced by the foregoing amendments to the specification.

Amendments to the Claims

Claims 1, 3-9, 11, 12, 28-33 and 35-48 were pending in the instant application as of the issuance of the present Office Action, and claims 2, 10, 13-27 and 34 were previously cancelled without prejudice. Claims 1 and 9 have been amended to incorporate the limitations of claims 4 and 36, respectively, solely in an effort to expedite the allowance of the application. Claims 3, 4, 35, 36 and 44 are hereby cancelled herewith without prejudice or disclaimer. Claims 37-40 and 45 have been amended to correct claim dependencies. Applicants reserve the right to pursue previously claimed subject matter in one or more continuing applications. No new matter has been introduced by the foregoing amendments.

With respect to the rejections maintained by the Examiner, Applicants respectfully request reconsideration and examination of this application and the timely allowance of the pending claims in view of the arguments presented below.

Rejection of claims 1, 3-9, 11, 12, 28-33 and 35-40 under 35 U.S.C. § 103(a)

The Examiner has maintained his rejection of claims 1, 3-9, 11, 12, 28-33 and 35-40 under 35 U.S.C. § 103(a) as being unpatentable over Tuschl *et al.* (US 2004/2059247 A1), Elbashir *et al.* (The EMBO Journal Vol. 20(23), 2001), Klug *et al.* (European Journal of Physiology, Vol. 441 (6 suppl 2): S27-S35, 1996), Brown *et al.* (WO 94/19493), Siddique *et al.* (Neurology Vol. 47 (suppl 2): S27-S35, 1996), and Kunst *et al.* (Nature Genetics Vo.. 15:91-94, (1996)). Specifically, the Examiner alleges that "[b]oth Tuschl et al and Elbashir have taught that siRNA discriminate and inhibit targets with as little as one nucleotide change and have also taught where in the siRNA molecule such changes can be made with the most effective selection of target."

Applicants respectfully traverse this rejection with respect to the amended claims, at least for the reasons set forth in Applicants' response filed February 11, 2009, which are partially reiterated below, and augmented with new arguments. As such, Applicants maintain their position that one skilled in the art would have had no reasonable expectation that single nucleotide discrimination between a wild type and mutant alleles of the SOD1 gene could be achieved using siRNA technology at the time the instant invention was made.

Applicants submit that Tuschl *et al.* and Elbashir *et al.* describe a single experiment[1] that is speculative at best and fails to demonstrate that single-nucleotide siRNA discrimination among two related alleles of the same gene was realistically achievable at the time of the invention. Importantly, the Tuschl/Elbashir experiment is a standard gene-specific silencing experiment, wherein sequence changes are introduced into the paired segments of siRNA duplexes to examine the effects of these sequence changes on the efficiency of silencing a single target gene sequence (a firefly luciferase reporter sequence). Both Tuschl et al. and Elbashir et al. report that "transversion of the AU base pair located opposite the predicted target RNA cleavage site or 1 nt further away from the predicted site prevented target RNA cleavage". However, aside from the single firefly luciferase target sequence, there is no indication that this siRNA retains the ablity to cleave other RNAs, including target RNAs to which it is perfectly matched. In fact, the Tuschl/Elbashir experiment provides no evidence that the introduction of a target mismatch in the center of the siRNA results in anything other than a "dead" or inactive siRNA. Accordingly, there is no reasonable basis for one of skill in the art to conclude that siRNA in the Tuschl/Elbashir experiment would be able to facilitate RNA interference of a mutant target allele while preserving the expression of a wild-type allele.

Moreover, as discussed in the previous response, one of skill in the art at the time the application was filed would have seriously doubted the applicability of the Tuschl/Elbashir experiment given the publication of several contemporaneous reports suggesting that siRNAs were in fact generally tolerant of sequence changes that introduce single-base mismatches between the siRNA and its target. For example, Boutla *et al.* (Boutla A *et al.*, *Current Biology,* 11: 1776-80 (2001), previously made of record) reported that siRNAs differing from the sequence of their target mRNA at one or more nucleotides retained efficacy, indicating that the siRNA technology did not require perfect sequence complementarity of the siRNA with the

[1] Applicants note that the Tuschl et al. patent publication and Elbashir et al. research article refer to the same experiment conducted by the same group of investigators.

mRNA to silence its expression. Specifically, a reference siRNA with full complementarity to *Notch* mRNA exhibited 93% penetrance (measure of silencing), while three different mutant siRNAs exhibited values of 81%, 88%, and 93% penetrance, leading Boutla *et al.* to conclude that "a perfect match to the target RNA is not necessary to initiate the RNAi response" (page 1779).

In the instant Office Action, the Examiner acknowledges the Boutla et al. reference but alleges that "the siRNAs of the reference were not designed as taught by the prior art". Applicants respectfully disagree. It should be noted that both the Tuschl/Elbashir experiment and the Boutla *et al.* experiment were designed to investigate how sequence changes within the oligonucleotide duplexes affected silencing/target recognition. Boutla *et al.* states "it was our intention to introduce a single nucleotide exchange that would interfere as much as possible with substrate binding" (see page 1779, column 1, paragraph 3, lines 2-4, emphasis added). Accordingly, Boutla *et al.* introduced a single, **centrally positioned mismatch** relative to the mRNA target sequence in each of the three mutant siRNA sequences (see Table 1(j), 1(k) and 1(l)). In fact, Boutla et al. introduced mismatches at the same position (P10) as the siRNAs in the Tuschl/Elbashir experiment. Nevertheless, despite the fact that the Boutla siRNAs were designed in the same fashion as the Tuschl/Elbashir siRNAs, the results were different. Accordingly, Applicants take the position that the Boutla *et al.* results are indicative of the unpredictable state of the art at the time of the instant invention, and reflect the unexpected nature of the invention.

Applicant finds additional evidence in the work of Holen *et al.* (Holen T. *et al.*, *Nucleic Acids Research,* 30(8): 1757-66 (2002), previously made of record) that the state of the art was unsettled at the time of the invention. Holen *et al.* synthesized siRNA to target sites within the mRNA of human tissue factor (TF), and observed that the wild-type siRNA *hTF167i-wt* exhibited 80% silencing capability. By comparison, the mutant siRNA *hTF167i*-M1 with a single-nucleotide **"central"** mismatch (see Figure 6A) "exhibited 65% silencing capability", leading the authors to conclude that "RNAi to a certain degree tolerates siRNA:mRNA mismatches" (page 1765). Thus, although the mismatch was chosen to be "maximally disruptive" as in the Tuschl/Elbashir experiment (page 1763, first column, second paragraph, lines 1-10) the authors demonstrated that it only partially reduced the rate and extent of target depletion (page 1765, column 1, paragraph 4, lines 12-15).

Further evidence of the unpredictable state of the art at the time of the invention is found in the work of Jacque *et al.* (Jacque, J-M. *et al.*, *Nature*, 418: 435-438 (2002), made of record in the present Amendment as **Exhibit A**), Yu *et al.* (Yu, J-Y. *PNAS*, 99: 6047-6052 (2002), made of record in the Supplemental Information Disclosure Statement filed on September 17, 2007) and Hamada *et al.* (Hamada, M., *Antisense and Nucleic Acid Drug Development*, 12: 301-309 (2002), made of record in the present Amendment as **Exhibit B**). Jacque *et al.* directed siRNA duplexes against several regions of the HIV-1 genome, including the viral long terminal repeat (*LTR*). *LTR* was targeted with both the wild-type siRNA *TAR* and the single-nucleotide mutant *MTAR*, both of which suppressed reverse transcription activity to nearly the same extent (page 435, Figure 1b). Similarly, Yu *et al.* observed that hairpin RNA (shRNA) possessing a single mismatch relative to the *luc-GFP* target silenced the reporter to nearly the same extent. Any difference was within the margin of error (page 6048, Figure 2c). Finally, Hamada *et al.* found that wild-type siRNA targetting *JDP-2* exhibited reporter silencing of 60%, while the single-nucleotide mutant siRNA silenced reporting by 30%. The authors comment that the limited RNAi effects observed by themselves, as well as Elbashir *et al.* "were not completely in accord with the findings of more recent studies, possibly because of the different conditions used" (page 305), thereby acknowledging the uncertainty prevailing in contemporary research.

Collectively, the preceding references suggest that the effect of single-nucleotide mismatches (including centrally-placed mismatches) was highly unpredictable at the time of the invention, and that, as a result, one skilled in the art would have had no reasonable expectation that allele-specific RNAi silencing of the mutant allele of the SOD1 target gene would be successful. As such, it would not have been obvious to one of skill in the art make the claimed invention with any reasonable expectation of success.

In view of the foregoing, Applicants request that the rejection under §103(a) be reconsidered and withdrawn.

Rejection of claims 41-48 under 35 U.S.C § 103(a)

The Examiner has maintained his rejection of claims 41-48 under 35 U.S.C. § 103(a) as being unpatentable over Tuschl *et al.* (US 20042059247), Elbashir (The EMBO Journal Vol. 20(23), 2001), Klug *et al.* (European Journal of Physiology, Vol. 441 (6 Suppl): R205, 2001), Brown *et al.* (WO 94/19493), Siddique *et al.* (Neurology Vol. 47 (suppl 2): S27-S35, 1996), and

Kunst *et al.* (Nature Genetics Vol. 15:91-94, (1996) as applied to claims 1-12 and 28-40 above, and further in view of Brummelkamp *et al.* (Science Express, 21 March 2002).

Here again the Examiner has relied on Tuschl *et al.* and Elbashir *et al.* as the primary references for the rejection of claims 41-48 under § 103. Applicants submit that the rejection is improper for at least the reasons discussed above with respect to the rejection of claims 1, 3-9, 11, 12, 28-33 and 35-40. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw his rejection of claims 41-48.

SUMMARY

In view of the above amendment and response, Applicants believe the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney, Debra J. Milasincic, Esq., at (617) 227-7400.

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Respectfully submitted,

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